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# Troglomorphism in the brittle star *Ophionereis commutabilis* Bribiesca-Contreras et al., 2019 (Echinodermata, Ophiuroidea, Ophionereididae)

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## Abstract

Due to their peculiar and sometimes bizarre morphology, cave fauna (across invertebrates and vertebrates from both aquatic and terrestrial cave habitats) have fascinated researchers throughout history. Despite their success in colonizing most marine ecosystems, the adaptations of cave brittle stars (Ophiuroidea) to a stygobiotic lifestyle have been scarcely examined. Employing comparative methods on a data set of two species belonging to the genus *Ophionereis*, this study addresses whether a cave-dwelling species from Cozumel exhibited similar troglomorphic traits as those of other taxa inhabiting caves. Our work demonstrated that some characters representing potential morphological cave adaptations in *O. commutabilis* were: bigger sizes, elongation of arms and tube feet and the presence of traits potentially paedomorphic. In addition, an element of ophiuroid's photoreceptor system, as well as pigmentation, was observed to be peculiar in this stygobiotic species, plausibly as a result of inhabiting a low light-energy environment. Finally, we add evidence to the statement that *O. commutabilis* is a cave endemic species, already supported by demography, distribution and origin of this species, and now by a typical array of troglomorphisms.

## Keywords

adaptation, Aerolito, anchialine system, cave, Cozumel, ophiuroid, stygobiotic

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## Introduction

Several traits are often associated with cave-dwellers, which are also known as troglomorphisms, defined as a morphological modification in cave fauna (Culver et al. 1995; Romero 2009). These modifications or morphological traits could be expressed as reductive or constructive traits, such as the lack of photoreceptors or depigmentation in the case of the former and as the hypertrophy of appendages for the latter (Mejía-Ortíz et al. 2006, Gonzalez et al. 2018). All these traits allow organisms to survive in caves, environments that constitute isolated and harsh habitats (Culver and Pipan 2009).

According to Bishop et al. (2015), anchialine caves are "a tidally-influenced subterranean estuary located within crevicular and cavernous karst and volcanic terrains that extends inland to the limit of seawater penetration". These environments are thought to be food-limited, with detritus and dissolved organic matter being the most important nutritional sources (Mejía-Ortíz et al. 2013). However, several caves have an important contribution of food from bacterial activity (Sarbu et al. 1996; Pohlman et al. 1997; Brankovits et al. 2017). The limitation of food is related to the fact that caves are completely devoid of sunlight and lack of sources of primary productivity (Culver et al. 1995; Gonzalez et al. 2018).

Although cave fauna is mainly composed by crustaceans, several studies have reported organisms of other taxa inhabiting these ecosystems (Sket 1996; Gibert and Deharveng 2002; Calderón-Gutiérrez et al. 2017). Concerning echinoderms, 58 species have been reported inhabiting cave environments, distributed into the five classes of the phylum: Crinoidea (2 species), Asteroidea (8), Ophiuroidea (39), Echinoidea (5) and Holothuroidea (4) (Martínez García et al. 2009; Pomory et al. 2011; Bribiesca-Contreras et al. 2013; Tan et al. 2014; Okanishi and Fujita 2019; Okanishi et al. 2019). However, only five of these species have been described as stygobiotic species (restricted aquatic cave-dwelling organism), the starfish *Copidaster cavernicola* Solís-Marín & Laguarda-Figueras, 2010 and the ophiuroids *Amphicutis stygobita* Pomory, Carpenter & Winter, 2011, *Ophiozonella cavernalis* Okanishi & Fujita, 2018, *Ophionereis commutabilis* Bribiesca-Contreras et al., 2019 and *Ophiopsila xmasilluminans* Okanishi, Oba & Fujita, 2019. This is surprising considering that there are over 7,500 species of echinoderms that live in a wide variety of habitats (Zhang 2011). Also striking is the fact that although Ophiuroidea is the largest class of echinoderms (about 2100 described species according to O'Hara et al. (2018)) and are generally photonegative (Stöhr et al. 2012; O'Hara et al. 2018), only a few (39) species have been found in caves.

Troglomorphism has been investigated mainly on arthropods and vertebrates from both aquatic and terrestrial caves (Poulson and White 1969; Mejía-Ortíz and Hartnoll 2005; White and Culver 2012; Mejía-Ortíz et al. 2013; Rizzato and Bichuette 2017). Despite the existence of some studies about troglomorphism on other taxa (White and Culver 2012; Gonzalez et al. 2018), no extensive study has been conducted on stygobiotic echinoderms. Instead, only a few remarks have been made on the morphology of species of echinoderms and its relation with the cave-habitat (Mejía-Ortíz et al. 2007;

Solís-Marín and Laguarda-Figueras 2010; Pomory et al. 2011; Brom et al. 2015; Carpenter 2016; Okanishi and Fujita 2018; Bribiesca-Contreras et al. 2019).

Thus, the aim of this study is the identification of morphological adaptations of *O. commutabilis*. To test the hypothesis that *O. commutabilis* shows troglomorphisms, we carried out a comparative study between *O. commutabilis* and its epigean congener *O. reticulata*. A comparison with other epigean congeners for several traits, as well as the similarities among cave-dwelling brittle stars are discussed. As this is one of the first studies to investigate troglomorphism in brittle stars, our assumptions are mainly based on the analogy to previously identified troglomorphic traits throughout other stygobiotic taxa. Implications for troglomorphism are discussed with respect to concepts of cave biology.

## Methods

### Species selection

*Ophionereis reticulata* (Say, 1825) was selected to use as a reference for comparison, based on both morphological and genetic resemblance between Caribbean *Ophionereis* species with *O. commutabilis* (Bribiesca-Contreras et al. 2013, 2019). Therefore, specimens of *O. commutabilis* ($N$ = 46) from the anchialine system El Aerolito, Cozumel Island (20°27'58.4"N, 86°58'41.2"W) (details in Bribiesca-Contreras et al. 2019) were compared with their reef relatives, *O. reticulata* ($N$= 59) from localities in Quintana Roo, Mexico. All the analysed material is deposited in the "Colección Nacional de Equinodermos Dra. María Elena Caso", at Instituto de Ciencias del Mar y Limnología, of Universidad Nacional Autónoma de México (**UNAM**).

### Selection of morphological traits

Morphological traits were chosen based on the previous studies on other taxa, and from our personal observations. We sampled and compared the morphological traits described below. A detailed discussion of each character is provided.

#### Arms and tube feet length

Elongation of body appendages are well documented traits in cave fauna, as they affect both sensorial and feeding structures in aquatic and terrestrial environments (Turk et al. 1996; Mejía-Ortíz et al. 2006, 2013). Arms of brittle stars are used mainly for locomotion and present tube feet and spines, both being the only known sensorial organs of this taxa (Hajduk 1992; Zueva et al. 2018). Spines and tube feet also participate in the acquisition of food by suspension feeding (Stöhr et al. 2012). Cave brittle stars show unique patterns of tube feet and arm length, proposed to be the result of differ-

ent forms of locomotion and feeding in these environments (Bribiesca-Contreras et al. 2019; Pomory et al. 2011).

Here we evaluate the elongation of arms and tube feet as a potential morphological adaptation to the cave. Measures of the structure's length were correlated with the disc diameter of each specimen. Length of arms was measured from the first to the last segment, considering only complete arms without regenerating scars. Tube feet and oral tentacle length were measured from the base (at the tentacle pore or tentacle basin, respectively) to the tip of the structure. Tube feet were considered from proximal, middle and distal portions of the arm (based on Munday (1993)), and were measured using image analysis software (ADOBE PHOTOSHOP CC: RRID: SCR_014199) calibrated with scaled photographs of fixed organisms.

**Regeneration frequency**

Regeneration is a common process on brittle stars, caused by damage of arms through sub-lethal predation. As a result, regeneration rate of arms is often used as an estimate of predation pressure (Sköld and Rosenberg 1996; Dupont and Thorndyke 2006; Yokoyama and Amaral 2010), which in caves decreases in importance or is absent (Gibert and Deharveng 2002). The regeneration frequencies of each specimen were registered by counting the regenerating scars on the oral side. To identify the scars, we compared differences in colour and size patterns between regenerated portions and old sections.

**Paedomorphic traits**

Morphological juvenile traits retained by sexually mature organisms have been reported for cave-dwellers, also known as paedomorphic features (Culver 1982; Sket 1996; Culver and Pipan 2009). Cave brittle stars could show some paedomorphic traits related to organization, size and calcification of skeletal plates of arms and disc (Pomory et al. 2011). For this reason, external morphology and microstructure of arm plates (dorsal, ventral, lateral, spines, tentacle scales) from proximal, middle and distal portions of the arm of mature and juvenile specimens were analysed. Likewise, radial shields and buccal skeleton (oral and adoral shields; oral and dental plates, as well as teeth) were considered.

The arm ossicles used for microstructure examination were obtained from one arm of each specimen selected. Tissue was removed by soaking two segments of each portion in 5% sodium hypochlorite and later washed with MilliQ water. Ossicles were mounted on a stub and coated with gold for taking micrographs using a Hitachi SU1510 scanning electron microscope (SEM).

Images of the external face of dorsal, ventral and lateral arm plates were analysed using both geometric morphometrics and morphological approaches. For geometric morphometrics, TPS, MAKEFAN8 and MORPHOJ *v*2.0 software (MorphoJ, RRID: SCR_016483) were used. Landmarks (LM) were digitized to describe the perimeter of the plates (Suppl. materials 1, Fig. S1). Madreporic oral shields, teeth, spines and tentacle scales were examined by comparing the morphology of outer face between

species. Oral, radial and adoral shields, as well as oral and dental plates, were analysed by comparing porosity of external stereom.

### Photoreceptors

Brittle stars have a photoreceptor system consisting of nerve bundles, chromatophores and expanded peripheral trabeculae (EPT) (Hendler and Byrne 1987; Aizenberg et al. 2001). EPTs are hemispheres on the outer face of arm plates and have been suggested to be a structural adaptation relating to chromatophore activity (Sumner-Rooney et al. 2018). Considering that caves exhibit total darkness, processes like photoreception and bioluminescence, as well as the involved structures, could be modified. Therefore, we estimated the EPTs density of the central region of the dorsal arm plates by using SEM images.

In addition to morphological traits, spectral transmittance of isolated dorsal arm plates was estimated by coupling a source of white light (LS-1-II Ocean Optics) to an HR4000 spectrophotometer. We used two optical fibres of 50 and 100 μm on different positions to obtain readings of three dorsal-proximal arm plates of each species (*O. reticulata* and *O. commutabilis*). Data were normalized and analysed on ORIGIN PRO 9.1 software (Origin, RRID: SCR_014212).

### Statistical analyses

We analyzed the number of arms and specimens regenerating as well as arm and tube feet ratios between the two species considered. Data and R-scripts used throughout this work are available on the supporting information. All analyses were performed under software RSTUDIO *v*3.5.0. (Team 2016).

Concerning geometric morphometrics analysis, after the Procrustes fit of the sample, canonical variate analysis (CVA) was performed to explore morphological differences among species using geometric morphometric analysis of arm plates. *P* values were calculated using a permutation test based on 100,000 iterations of the Mahalanobis distances for differences. CVA, including estimation of the significance of Mahalanobis distances using a parametric approach (Procrustes ANOVA), was also performed to assess the statistical robustness of the groups delineated in the CVA.

## Results

### Appendages length

A remarkable arm elongation is observed in *O. commutabilis*, with arms up to 20 times the disc diameter and a mean of 13.2 in comparison to 6.6 of *O. reticulata*. This ratio showed statistical differences between species (ANOVA, $F_{1, 105}=559.3$; $p<0.001$). Furthermore, we found that arms of specimens of equal size from both cave and epigean

species are integrated by segments of similar length (Suppl. materials 2, Table S1), hence, cave species having more segments. No differences of oral tentacle nor tube feet length were found among species in either proximal or middle portions of arms. Distal tube feet showed statistical differences in length (ANOVA, $F_{1, 48}$=18.1; p <0.001), where the stygobiotic specimens had longer tube feet than *O. reticulata*. These results are summarized in Table 1.

After measuring the disc diameter, we observed that stygobiotic specimens showed bigger sizes. For the disc diameter, we observed between mature *O. reticulata* and *O. commutabilis*, statistical differences (ANOVA, F1, 88= 4.54; p <0.05). Cave-dwelling specimens having a disc diameter 14% bigger than its epigean congener.

## Regeneration frequency

Based on recorded observations of specimens of *O. commutabilis*, 32% (n= 46) showed signs of arm regeneration. Besides, 14% of arms observed (n= 196) were damaged. In comparison, from the 51 individuals of *O. reticulata* analyzed, 70% showed signs of arm regeneration. Correspondingly, 35% of arms (n= 213) were damaged in specimens of *O. reticulata*.

The average of arms regenerating per individual in cave-dweller specimens was 0.7 (14.42%) while in reef individuals was 1.6 arms (33.16%). Statistical analysis showed a difference between species for these data (ANOVA, $F_{1, 95}$= 12.52; p <0.001).

## Microstructures

Dorsal arm plates (DAP) of both species are different in form, being hexagonal in mature *O. commutabilis* and trapezoid in *O. reticulata* (Fig. 1). Meanwhile, DAP of juvenile specimens of *O. reticulata* are hexagonal (Fig. 1c). Geometric morphometric analyses allowed us to confirm the statistical significance of differences in form between plates of mature specimens of both species (Table 2). Deformation grids in the DAP shape showed the mentioned change based on LM displacement vectors (Fig. 1d). Although ventral arm plates (VAP) of *O. reticulata* are slightly wider than those of *O. commutabilis*, the general shape is similar in both species (Fig. 2). Ventral arm plates (VAP) of mature *O. reticulata* specimens are also wider than juveniles of this species (Fig. 2c). Differences in form of VAP between species were confirmed with CVA ($P < 0.001$) and deformation grid exhibited the difference in width among species (Fig. 2d). Both DAP and VAP of juvenile specimens of *O. commutabilis* show the same shape than plates of mature specimens. Finally, lateral arm plates showed significant differences in form between species (Table 4) but no clear pattern associated (Fig. 3). Examination of arm plates allowed us to corroborate the correlation between serial and ontogenetic variation, that is to say, distal plates analogous to plates of juvenile specimens.

**Table 1.** Summary of morphometric results showing significant (S) and not significant (NS) differences for each trait. For traits with statistically significant differences the result of it with respect to the cave species is expressed as longer/bigger than *O. reticulata* (↑). NT: not statistically tested.

| Trait | Differences | Result |
|---|---|---|
| Arm length | S | *O. commutabilis* ↑ |
| Number of arm segments | NT | *O. commutabilis* ↑ |
| Oral tentacle length | NS | - |
| Proximal tube feet length | NS | - |
| Middle tube feet length | NS | - |
| Distal tube feet length | S | *O. commutabilis* ↑ |

**Table 2.** Geometric morphometrics results for DAP. *Ophionereis* spp. dorsal arm plates Procrustes ANOVA and P values based on permutation test results with environment (Reef for *O. reticulata* and Cave for *O. commutabilis*) as covariate (Mahalanobis distance in parenthesis).

| Procrustes ANOVA | | | | | |
|---|---|---|---|---|---|
| **Effect** | **SS** | **MS** | ***df*** | ***F*** | ***P*(param.)** |
| Individual | 0.06245164 | 0.0052043037 | 12 | 3.01 | 0.0005 |
| Residual | 0.53979931 | 0.0017301260 | 312 | | |
| ***P* value based on permutation test** | | | | | |
| | Environment | | | Cave | |
| | Reef | | | <0.0002(2.39) | |

**Table 3.** Geometric morphometrics results for VAP. *Ophionereis* spp. ventral arm plates Procrustes ANOVA and P values based on permutation test results with environment (Reef for *O. reticulata* and Cave for *O. commutabilis*) as covariate (Mahalanobis distance in parenthesis).

| Procrustes ANOVA | | | | | |
|---|---|---|---|---|---|
| **Effect** | **SS** | **MS** | ***df*** | ***F*** | ***P*(param.)** |
| Individual | 0.02209517 | 0.0012275093 | 18 | 5.00 | <0.0001 |
| Residual | 0.11482214 | 0.0002453465 | 468 | | |
| ***P* value based on permutation test** | | | | | |
| | Environment | | | Cave | |
| | Reef | | | <0.0001(7.34) | |

**Table 4.** Geometric morphometrics results for LAP. *Ophionereis* spp. lateral arm plates Procrustes ANOVA and P values based on permutation test results with environment (Reef for *O. reticulata* and Cave for *O. commutabilis*) as covariate (Mahalanobis distance in parenthesis).

| Procrustes ANOVA | | | | | |
|---|---|---|---|---|---|
| **Effect** | **SS** | **MS** | ***df*** | ***F*** | ***P*(param.)** |
| Individual | 0.11434060 | 0.0043977153 | 26 | 7.58 | <0.0001 |
| Residual | 0.73910392 | 0.0005801444 | 1274 | | |
| ***P* value based on permutation test** | | | | | |
| | Environment | | | Cave | |
| | Reef | | | <0.0001(6.27) | |

Concerning microstructures qualitatively analyzed, *O. commutabilis* tentacle scales are ovoid and longer than wide, which resembles the same plates of juvenile *O. reticulata* (Fig. 4a–c). On the other hand, spines showed no clear differences in form among species. All examined microstructures exhibited differences in porosity, with

**Table 5.** Comparison of several traits between a stygobiotic *Ophionereis* species and its closest epigean relatives. dd: disc diameter; al: arm length; DAP: dorsal arm plate; VAP: ventral arm plates; ND: No data.

| Species | Habitat | dd (mm) [Max] | Avg ratio (al/dd) [Max] | Avg segment length (mm) | Regeneration frequency (%) [for arms] | DAP shape | VAP shape | Reference |
|---|---|---|---|---|---|---|---|---|
| *Ophionereis commutabilis* | Cave-dwelling | 11.4 [17] | 13.2 [20] | 0.58 | 32 [14] | Hexagonal | Longer than wide | Present study |
| *O. reticulata* | Epigean | 11.4 [15] | 6.6 [8] | 0.55 | 70 [35] | Trapezoid | Quadrangular | Bribiesca-Contreras et al. 2013, Hendler et al. 1995, Present study |
| *O. vittata* | Epigean | 6.7 [10] | 8 [13] | ND | ND | Rounded hexagonal | Bell shaped | Bribiesca-Contreras et al. 2013, Hendler et al. 1995, Present study |
| *O. squamulosa* | Epigean | 5 [6] | 8 [ND] | ND | ND | Rounded hexagonal | Bell shaped | Bribiesca-Contreras et al. 2013, 2019, Pomory 2007 |
| *O. olivacea* | Epigean | 4.5 [6] | 5 [ND] | ND | ND | Roughly hexagonal | Bell shaped | Bribiesca-Contreras et al. 2013, 2019, Hendler et al. 1995 |

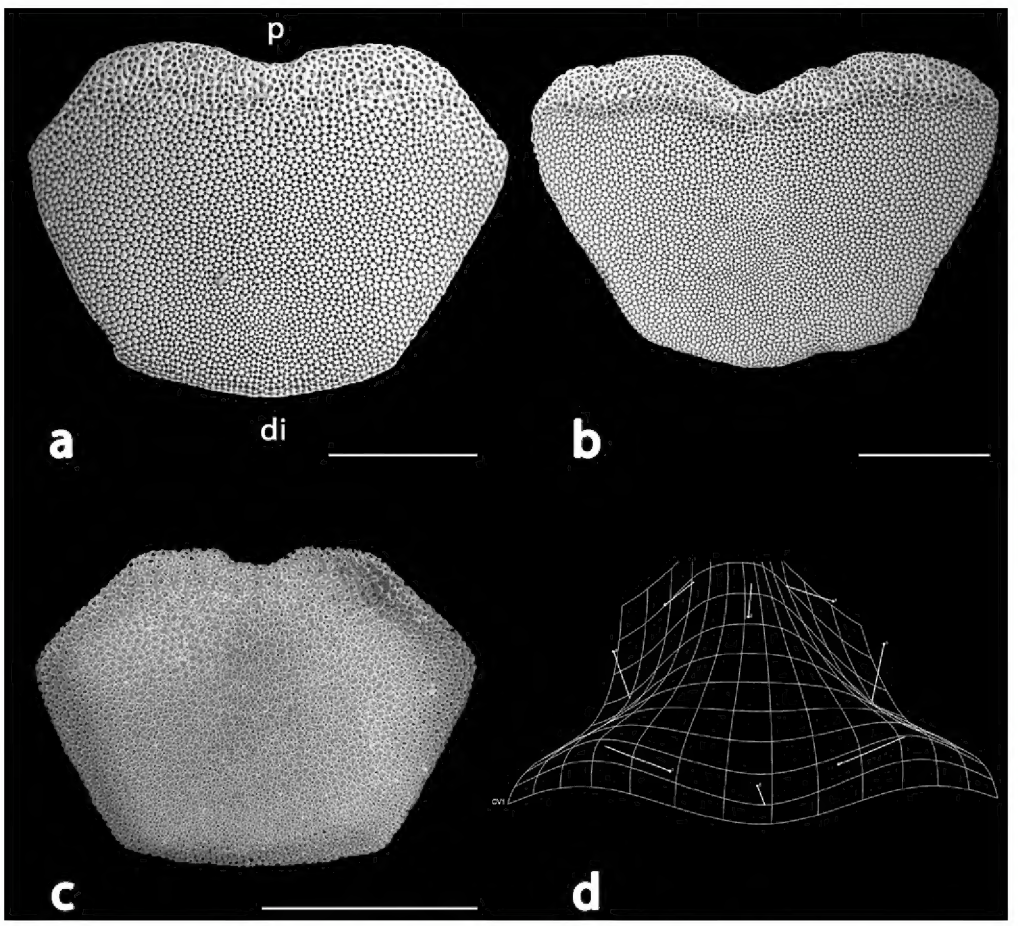


**Figure 1.** Scanning electron micrograph (SEM) of dorsal arm plates from mature *Ophionereis commutabilis* (**a**) and *O. reticulata* (**b**). SEM of dorsal arm plate of juvenile *O. reticulata* (**c**). Deformation grid of DAP shape showing deformation vectors (**d**). Orientation (p: proximal, di: distal). Scale bars: 400 μm.

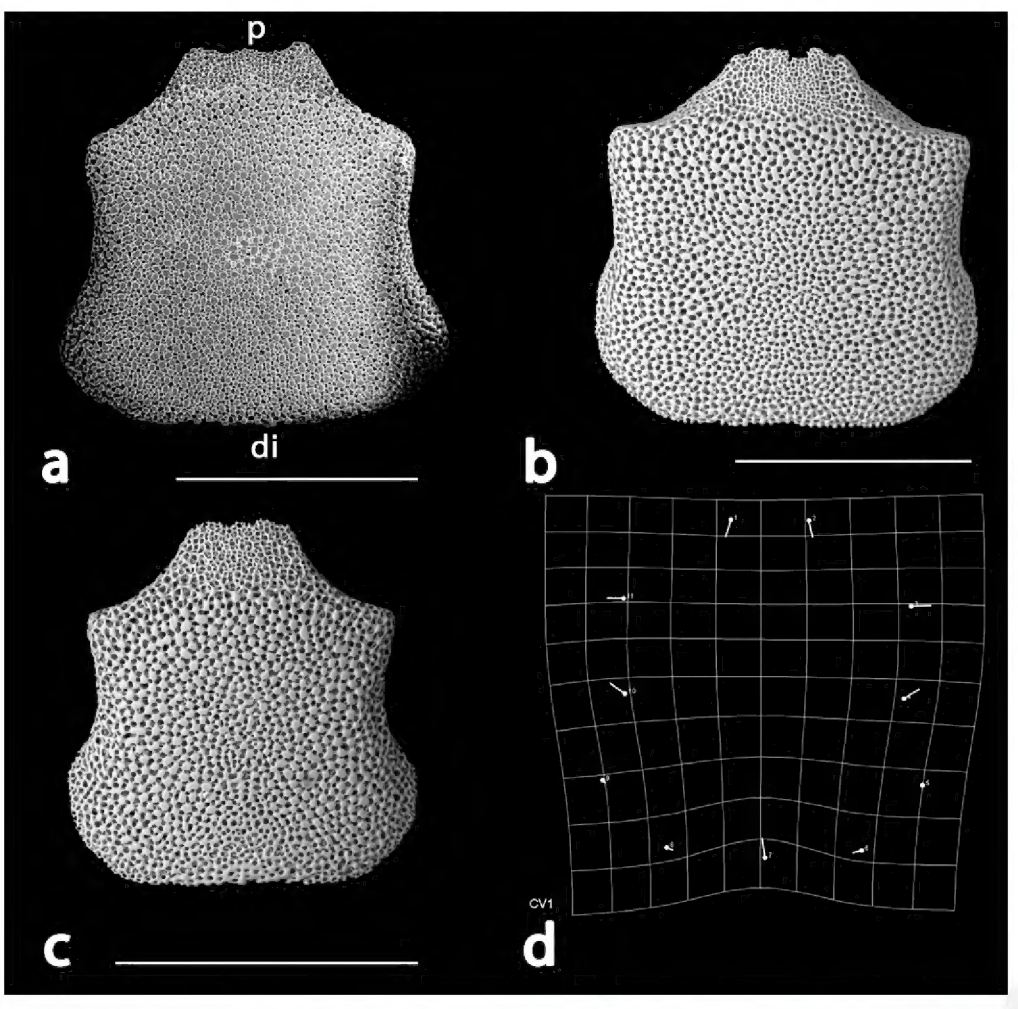


**Figure 2.** Scanning electron micrograph (SEM) of ventral arm plates from mature *Ophionereis commutabilis* (**a**) *and O. reticulata* (**b**). SEM of ventral arm plate of juvenile *O. reticulata* (**c**). Deformation grid of VAP shape showing deformation vectors (**d**). Orientation (p: proximal, di: distal). Scale bars: 500 μm.

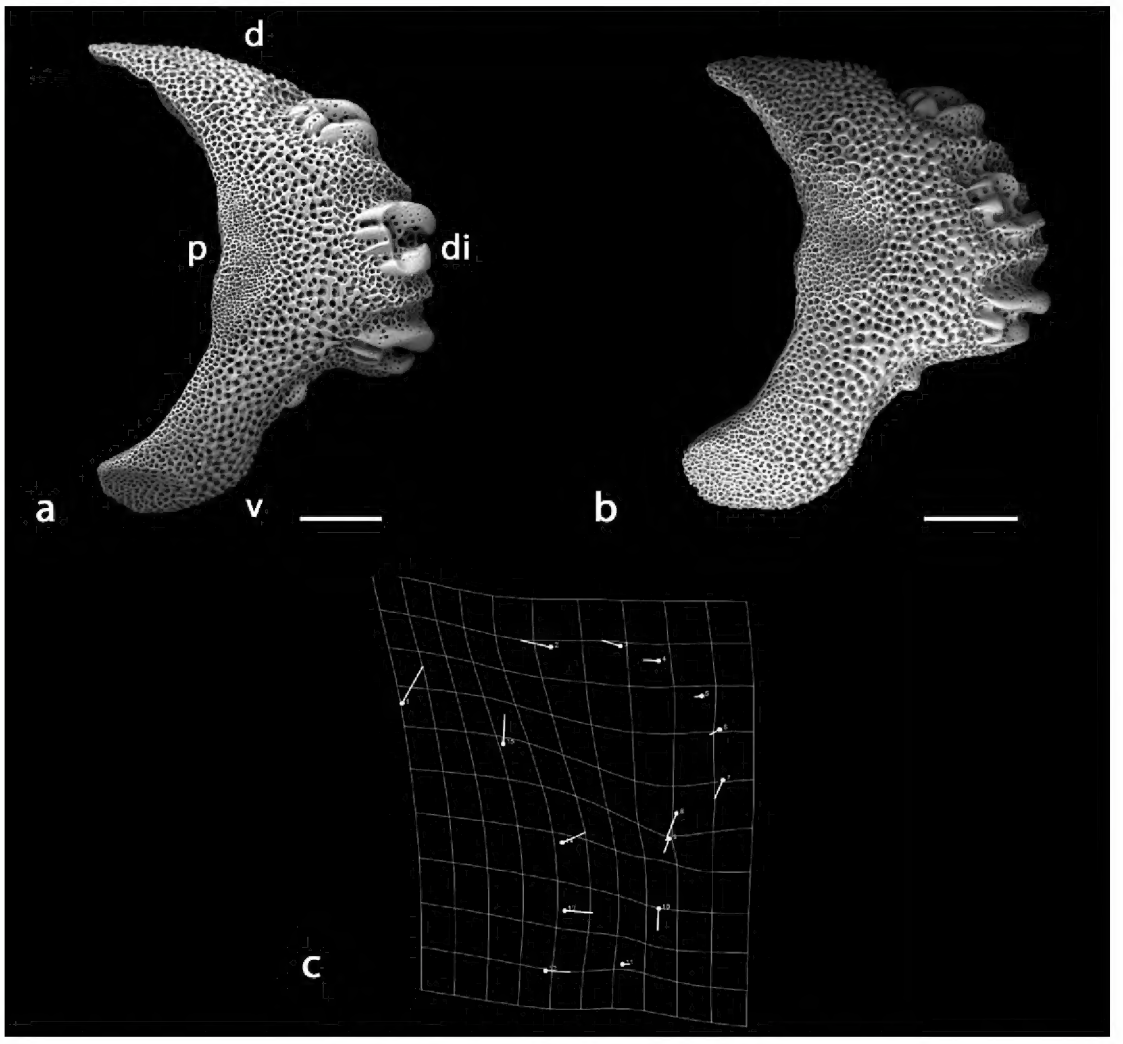


**Figure 3.** Scanning electron micrograph (SEM) of lateral arm plates from mature *Ophionereis commutabilis* (**a**) and *O. reticulata* (**b**). Deformation grid of lateral arm plate shape showing deformation vectors (**c**). Orientation (p: proximal, di: distal, d: dorsal, v: ventral). Scale bars: 200 μm.

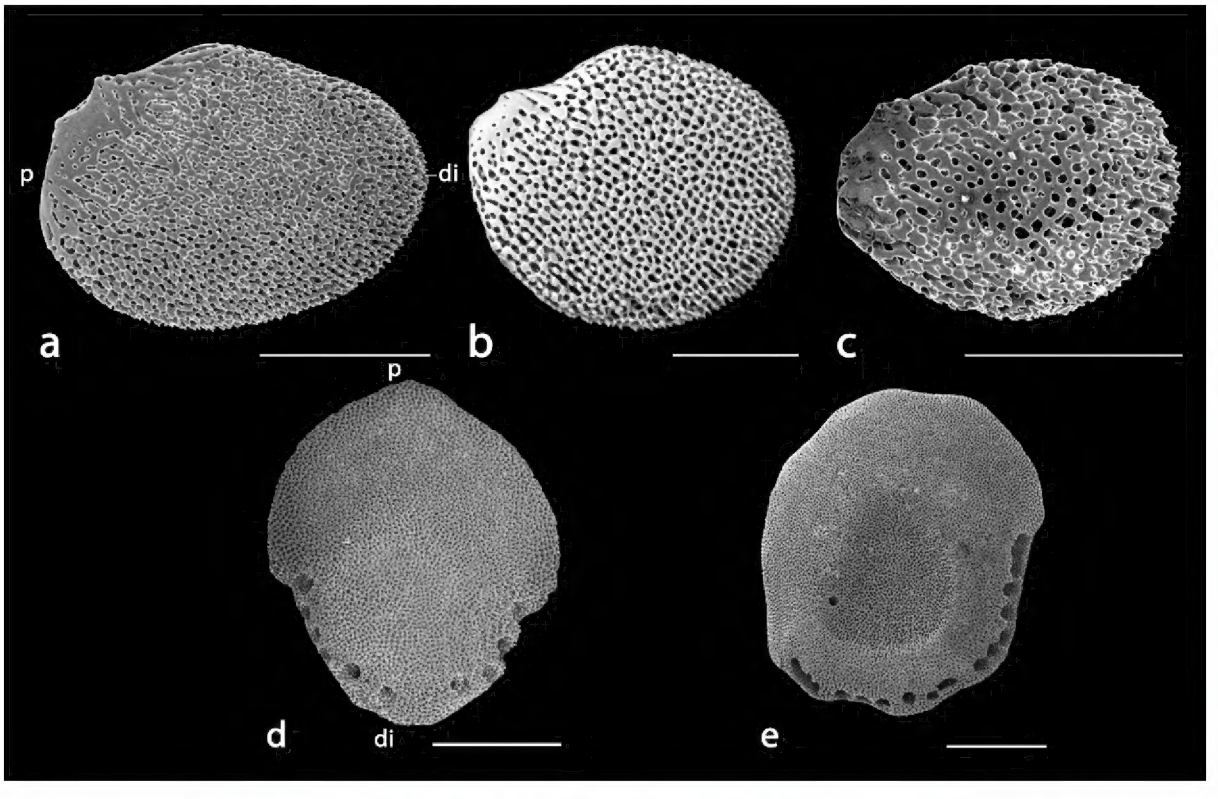


**Figure 4.** Scanning electron micrograph (SEM) of tentacle scales from mature *Ophionereis commutabilis* (**a**) *and O. reticulata* (**b**). SEM of tentacle scale of juvenile *O. reticulata* (**c**). SEM of madreporite of *O. commutabilis* (**d**) and *O. reticulata* (**e**). Orientation (p: proximal, di: distal). Scale bars: 200 μm (**a**–**c**); 500 μm (**d**–**e**).

the ossicles of the stygobiotic specimens being slightly more porous than its epigean congener (e.g. 5 to 9 pores surrounding each EPT in *O. commutabilis* and 4–7 in *O. reticulata* as shown in Figs 1, 5). Although these differences in porosity are observed in all ossicles, it is especially remarkable in distal arm plates, spines, tentacle scales and dental plates (Fig. 4 and Suppl. materials 1, Fig. S2). Madreporites between both species differ in form and in the number of hydropores (around eight in *O. commutabilis* and 17 in *O. reticulata*) (Fig. 4d–e). Lastly, though teeth showed great intraspecific variation, a clear pattern allowed us to distinguish between species. While *O. reticulata* possesses compound teeth, *O. commutabilis* reveals both uniform and compound teeth (Suppl. materials 1, Fig. S3).

## Photoreceptors

Dorsal arm plates (DAP) of *O. commutabilis* exhibit a pattern of EPTs agglomeration, however, this pattern is only present on some DAP from the distal portion of the arm of *O. reticulata* (Fig. 5a–c). This pattern decreases the EPT density (increasing its size) on the stygobiotic specimens in comparison to its epigean congener. The EPT density of DAP relativized with the disc diameter in *O. reticulata* (498 EPT/mm$^2$) almost doubled that of *O. commutabilis* (251 EPT/mm$^2$).

Differences in the arrangement and size of the EPT in both species corresponds to a different pattern of the inner stereom through a cross-section. DAP of *O. commuta-*

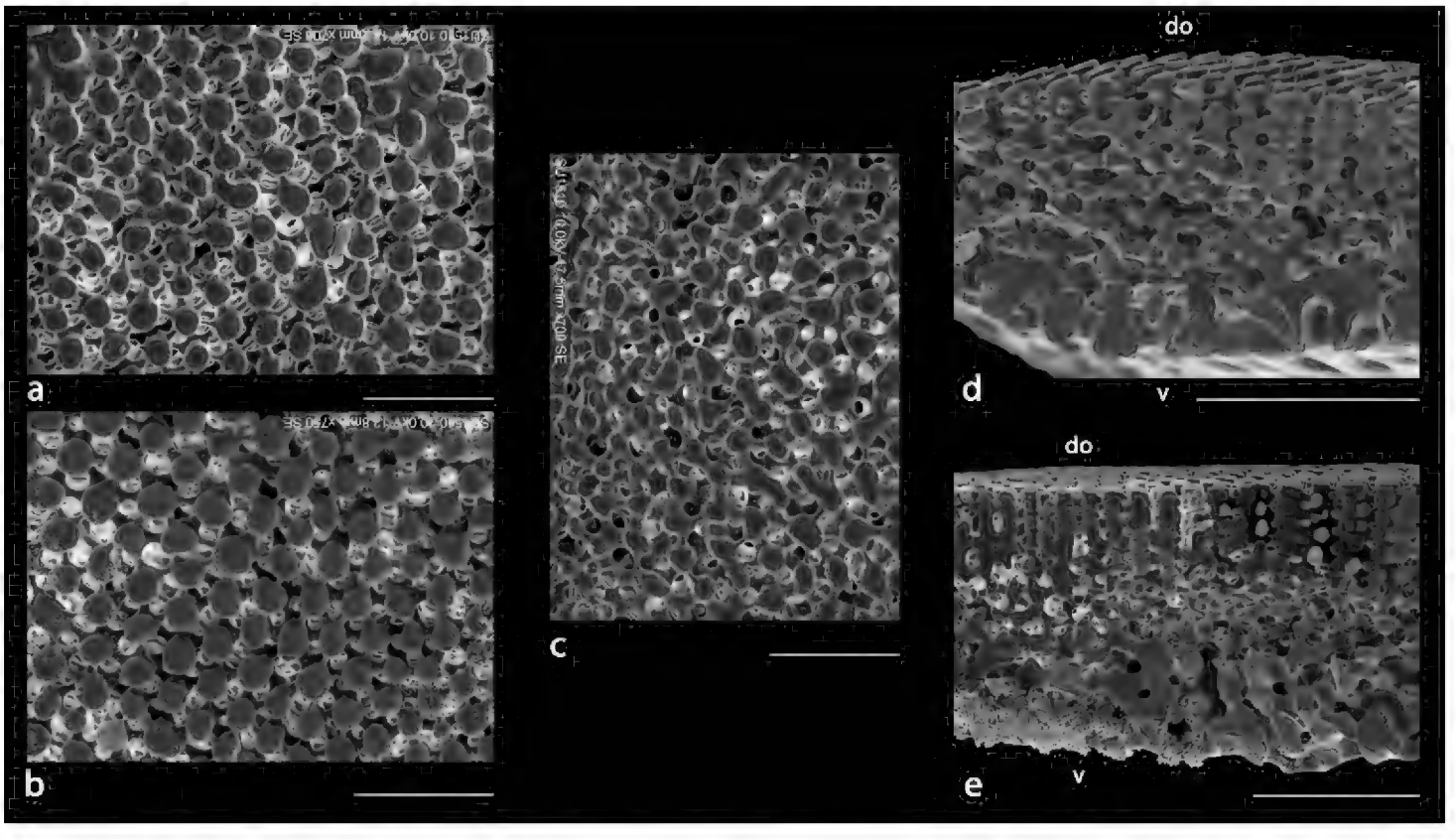


**Figure 5.** Scanning electron micrographs (SEM) of central region of dorsal arm plates (DAP) of *Ophionereis* brittle stars. SEM of a DAP from mature *Ophionereis commutabilis* (**a**) and *O. reticulata* (**b**). SEM of a DAP of juvenile *O. reticulata* (**c**). SEM of a cross-section of a fractured DAP from mature *O. commutabilis* (**d**) and *O. reticulata* (**e**). Orientation (do: dorsal, v: ventral). Scale bars: 50 μm (**a**–**c**); 100 μm (**d**–**e**).

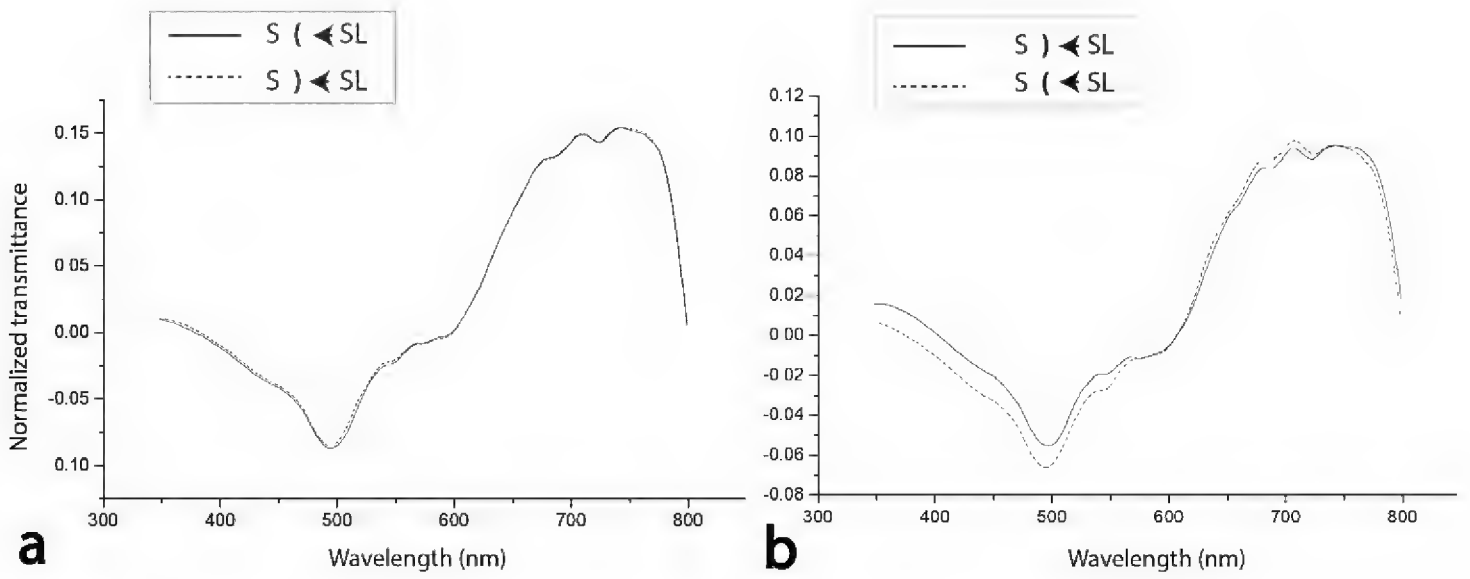


**Figure 6.** Dorsal arm plates transmittance of *Ophionereis reticulata* (**a**) and *O. commutabilis* (**b**). Two different arrangements between spectrophotometer (S), source light (SL) and plates are presented with concave section representing the inner face and convex the outer face ])-([.

*bilis* show a nearly uniform disorganized pattern across the plate, while DAP of the epigean specimens exhibit two clear patterns depending on the side of the plate: (1) a dorsal half with an organized stereom associated with EPT and (2) a ventral half with unsystematic stereom (Fig. 5d–e).

Correspondingly with the differences on the microstructure of DAP, transmittance values of DAP showed differences among species. The values of epigean specimens did not differ depending on the position of optical fibres. Meanwhile, differences were observed along the spectra between the two arrangements in the transmittance values of DAP of *O. commutabilis*. For both species, the transmittance was lower around 500 nm and greater for wavelengths between 600 and 780 nm (Fig. 6). In general, plates of stygobionts showed slightly lower values of transmittance than those isolated from epigean specimens.

## Discussion

Using a comparative morphological approach, we provide evidence of cave adaptation in *O. commutabilis*. This study quantified the main morphological differences observed between *O. commutabilis* and its epigean congener *O. reticulata*. Our findings were similar to those known in arthropods and are characteristic of changes considered as troglomorphism (Culver et al. 1995; Pérez-Moreno et al. 2016; Gonzalez et al. 2018).

Arm length is the most conspicuous trait of *O. commutabilis*, here we provide, for the first time, direct and quantitative evidence of this. As previously mentioned, elongation of body appendages is well documented in cave fauna as a troglomorphic trait, hence, arm elongation represents a potential morphological cave adaptation in *O. commutabilis*. The larger number of segments in *O. commutabilis* suggests that elongation is the result of having more segments than its epigean congener. Each segment has two

tentacle pores, thus cave-dwelling individuals have more tentacle pores and therefore more tube feet, as well as more spines. It has been proposed that appendage elongation increases the ability of cave fauna to locate food, avoid predation or improve sensory capability (Iliffe and Kornicker 2009; Mejía-Ortíz et al. 2006, 2013). Given that both spines and tube feet are important structures in sensory reception of ophiuroids (Hajduk 1992; Zueva et al. 2018), our results are relevant in giving a plausible explanation about the origin and function of adapted elongated arms. Additionally, these results confirm quantitively the first report of a species of Ophionereididae having arms that long (Stöhr et al. 2012; Bribiesca-Contreras et al. 2019) in average and as maximum (Table 5). This is valid not only for Caribbean species but also for species from the Pacific Ocean (e.g. *Ophionereis variegata* with an arm length average of five times the size of the disc or *O. amoyensis* with arms ten times the size of the disc) (Clark 1953).

Distal tube feet play an important role on detritus and suspension feeding (Warner 1982), which could explain *O. commutabilis* having longer distal tube feet than *O. reticulata*, suggesting that detritus and suspended particles are also (Mejía-Ortíz et al. 2013) an important source of food in the cave El Aerolito. On the other hand, oral tentacle, as well as proximal and middle tube feet, are commonly less specialized (Keogh and Keegan 2006), which provides an explanation for cave species not having longer structures than epigean congeners.

The tube feet, as well as their relation with arms in cave ophiuroids, need to be studied more deeply, as these structures probably play an important role in the evolution of cave ophiuroids. This is suggested by the striking differences in length and function among cave species and their epigean congeners demonstrated in *A. stygobita* (Carpenter 2016; Pomory et al. 2011), *O. xmasilluminans* (Okanishi et al. 2019) and *O. commutabilis* (Table 5), and between the cave species itself. Concerning arm length, the results obtained in this study are similar to that observed in *O. xmasilluminans* (Okanishi et al. 2019) and contrast with those observed in *A. stygobita* which have shorter arms (Pomory et al. 2011). Given that abiotic conditions are expected to be similar in the three caves, these differences could support the theory that elongation is associated with food-finding abilities or colonization times rather than specific abiotic factors (Delić et al. 2016).

Our results allowed us to confirm that *O. commutabilis* is distinguished by reaching big sizes in comparison to other Caribbean species of the genus (Bribiesca-Contreras et al. 2019) (Table 5). This is striking on a cave species, given that usually this fauna has a reduction in size as a response to a diminished food supply or quality (Culver et al. 1995; Culver and Pipan 2009). This could respond to the low predation rate expected for a cave environment (Gibert and Deharveng 2002) and the high population density of *O. commutabilis* in El Aerolito (Calderón-Gutiérrez and Solís-Marín 2014), factors that have been proposed to favor the survival of bigger organisms (Gage 1990; Bishop and Iliffe 2009). Larger bodies could also be advantageous in terms of energy economy and fecundity, important aspects in a cave (Trontelj et al. 2012). Interestingly, two species inhabiting similar environments show opposite patterns of population densities (low in *A. stygobita* and high in *O. commutabilis*) (Carpenter 2016) and relative sizes (small in *A. stygobita* and big in *O. commutabilis*) with respect to their epigean congeners.

The contrasting results of this study (*O. commutabilis*) compared with those obtained by Pomory et al. (2011) in *A. stygobita* point out the need for more studies on stygobiotic echinoderms. This will improve our understanding of troglomorphisms in the phylum and it would provide useful information to understand the evolution of marine organisms in caves.

Regeneration frequency was lower in *O. commutabilis* (32% of specimens and 14% of arms) than *O. reticulata* (70% and 35%). *O. commutabilis* shows similar percentages to *A. stygobita* (35% and 14%) (Carpenter 2016), another cave species. On the other hand, the regeneration frequency of *O. reticulata* is similar to that observed in other populations of this species and other epigean species (Lawrence and Vasquez 1996; Yokoyama and Amaral 2010). Therefore, the low incidence of damage in *O. commutabilis* indicates that sub-lethal predation is low inside El Aerolito as expected. This could be related with specimens inside the cave being usually found exposed rather than hidden (Bribiesca-Contreras et al. 2019: supplementary video 3), corresponding to the reduced escape behaviour observed on cave fauna (White and Culver 2012).

Stereom organization and porosity is the most conspicuous trait potentially considered as paedomorphic, being more porous in all the analysed ossicles of the cave species. Similar patterns have been proposed as an adaptive trait for deep-sea and cave brittle stars, improving feeding and gas exchange mechanisms and enhancing chemo and mechanoreception in harsh environments (Pomory et al. 2011; Carpenter 2016; Stöhr and Martynov 2016). We suggest that tentacle scales, DAP and VAP shape of *O. commutabilis* are paedomorphic traits based on the resemblance between mature cave specimen's ossicles and that of juvenile *O. reticulata*. Moreover, Bribiesca-Contreras et al. (2019) described conspicuous primary plates as a usual trait of *O. commutabilis*, which has been suggested as a paedomorphic trait in ophiuroids (Pomory et al. 2011; Martynov et al. 2015). Therefore, it seems evident that like cave brittle stars from Bahamas (Pomory et al. 2011), *O. commutabilis* specimens show paedomorphic traits on skeletal plates, as previously reported for the size in *O. olivacea* (Byrne 1991). It must be noted that *O. commutabilis* does not show some typical paedomorphic traits such as segment length-to-width ratio close to 10 (Stöhr and Martynov 2016). Moreover, some of the traits here reported as paedomorphic could be the result of other factors affecting the biology of this stygobiotic species (e.g. stereom development could be explained by abiotic factors in the cave). However, it must be considered that there are different levels of heterochronic changes affecting ophiuroids, which implies different levels of difficulty to recognize this characters, as well as different evolutionary effects on the species (Stöhr and Martynov 2016). The resemblance in DAP and VAP shape between *O. commutabilis* and the other Caribbean species (Table 5) should be studied more deeply to understand the evolutionary patterns of this structures in the group as well as the impact of a harsh environment such as caves in this. More species should be compared concerning the potentially paedomorphic traits presented in this work to define whether *O. commutabilis* present paedomorphosis.

The number of hydropores is equal among species of highly different geographical distribution, but inhabiting similar environments as *O. reticulata* and *O. schayeri* (Ezhova et al. 2016). However, that number is very different between closely related

species *as O. reticulata* and *O. commutabilis*, which inhabit very dissimilar environments. This is consistent with the hypothesis claiming that madreporites are ecologically informative (Ezhova et al. 2016). The differences among species in the type of teeth are relevant if we consider that teeth types have been used as an indicator of feeding preferences (uniform teeth for macrophagous feeders and compound for microphagous) (Medeiros-Bergen 1996; Brogger et al. 2015). Five ophionereidids have been reported to possess only compound teeth (Medeiros-Bergen 1996), while *O. commutabilis* shows both compound and uniform teeth, which could represent an adaptation, increasing the importance of omnivorous feeding in this cave system (as suggested by Bribiesca-Contreras et al. (2019) for other food sources). An alternative explanation for the presence of uniform teeth in *O. commutabilis* could be that they represent underdeveloped teeth, either for being ventral (the youngest according to Hendler (2018) or for the slow growth rate of cave organisms (Carpenter 2016). Though all the teeth were obtained from the ventral and dorsal portions of specimens of similar size, this explanation (being underdeveloped teeth) must be considered in further studies.

The presence of EPTs on DAP is conspicuous across the genus *Ophionereis*, not only in Caribbean species like *O. reticulata* but also in the Indo-Pacific species *O. porrecta* and *O. degeneri* (figures 15c and 16c in: Stöhr (2011)). Furthermore, based on figures from available literature, ophionereidids have higher EPT densities than species of the genus *Ophiocoma* (e.g. 15 vs 498) (Hendler and Byrne 1987) and *Ophiopsila* (e.g. 96 vs 498) (Deheyn et al. 2015). Though *O. commutabilis* shows expanded peripheral trabeculae on dorsal arm plates, these structures show a different pattern that decreases in density in contrast to its epigean congeners. These differences could suggest the effects of low energy and darkness typical of caves, in the photoreceptor system of this species. Moreover, the cave specimens DAP show a disorganized inner mesh, further suggesting stereom organization being reduced as an expensive or dispensable trait, as proposed for cave-dwelling taxa (Klaus et al. 2013). Concerning inner stereom it is especially relevant since it affects chromatophore activity (Hendler and Byrne 1987; Sumner-Rooney et al. 2018), suggesting an important impact of darkness in *O. commutabilis* life as previously discussed for the diversity of colouration of this species (Bribiesca-Contreras et al. 2019).

Stereom of cave specimens are similar to that of deep-sea ophiuroids in having less defined expanded peripheral trabeculae (Hendler and Byrne 1987), both environments having similar conditions (e.g. absence of light and low energy). On the other hand, agglomeration of EPT has been reported for bioluminescent species, particularly conspicuous in *Ophiopsila californica* (Deheyn et al. 2015). In addition, transmittance differences (due to whether the light beam passes through EPT first or last) on DAP of the cave species are similar to differences observed among bioluminescent (*Ophiopsila californica and Amphipholis squamata*) and non-bioluminescent (*Amphipholis pugetana*) species (Deheyn et al. 2015). Therefore, our results suggest that both photoreception and bioluminescence processes have affected the evolution of this species and confirm that differences in morphology of DAP between both species have an impact on light transmittance.

The lower transmittance peak observed in the two analyzed species corresponds to wavelengths that activate phototaxis in ophiuroids (Delroisse et al. 2016). This

adds evidence to the hypothesis that this species diverged from a shallow water lineage (Bribiesca-Contreras et al. 2019). On the other hand, the highest peak is usual in ophiuroids, since it corresponds to wavelengths attenuated on the first 10-20 cm depth (Deheyn et al. 2015). Finally, these results highlight the necessity of more studies to understand if the phototaxis in ophiuroids is related to their relative success in caves (in comparison with the other classes of the phylum).

Cave fauna shows particular morphological traits that could be considered to be troglomorphisms if they allow organisms to successfully colonize these harsh environments (Culver and Pipan 2009; Gonzalez et al. 2018). This paper demonstrates that brittle stars inhabiting an anchialine cave in Cozumel show troglomorphic traits such as arm (through the adding of segments) and distal tube feet elongation, increase in size, and possibly paedomorphic traits. This is, to our knowledge, the second study investigating troglomorphic adaptations in a group of echinoderms; our work confirms *that O. commutabilis* can be characterized as a stygobiotic species as demography, distribution and origin of this species suggested (Bribiesca-Contreras et al. 2013, 2019).

## Conclusion

In conclusion, troglomorphic traits of *Ophionereis commutabilis* include elongation of arms (as a result of the addition of segments) and increased sizes, similar to those observed for other cave fauna. Additionally, potentially paedomorphic traits are reported for an ophionereidid. Finally, the morphology of *O. commutabilis* confirms it as a stygobiotic species as demography, distribution and origin of this species previously suggested.

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## Supplementary material 1

**Supplementary figures S1–S3**

Authors: Francisco Márquez-Borrás, Francisco A. Solís-Marín, Luis M. Mejía-Ortiz

Data type: multimedia

Explanation note: **Figure S1.** Sets of Landmarks (LM) configurations designed to register the shape of the dorsal (A), ventral (B) and lateral (C–D) arm plates. Orientation (p: proximal, di: distal, do: dorsal, v: ventral). **Figure S2.** Scanning electron micrograph (SEM) of dental plates. SEM of dental plates from *Ophionereis commutabilis* (a-b) (modified from Bribiesca-Contreras et al. 2019) and *O. reticulata* (c-d), showing external (a, c) and internal faces (b, d). Scale bars: 500 μm. **Figure S3.** Scanning electron micrograph (SEM) of teeth from *Ophionereis commutabilis*, showing compound (a) and uniform teeth (b). Compound teeth from *O. reticulata* (c-d). Scale bars: a-c, 300 μm; c-d, 400 μm.

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## Supplementary material 2

**Supplementary table S1**

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Data type: table

Explanation note: Arm features of the stygobiotic species (*O. commutabilis*) and its epigean congener (*O. reticulata*).

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## Supplementary material 3

**R-scripts**

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Data type: statistical data

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